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WASHINGTON UNIV SEATTLE
WATER CONTENT OF STRATUM CORNEUM IN VIVO. (U)
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DADA17-72-C-2103

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Washington, D.C. 20314

⑨ SUBJECT: Annual Progress Report, 1 April 1972 -
31 March 1973

⑥ PROJECT: Water Content of Stratum Corneum *in vivo*.

⑮ CONTRACT #: DADA 17-72-C-2103

CONTRACTOR: University of Washington
Seattle, Washington 98195

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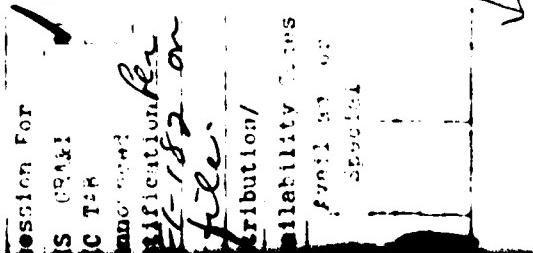
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I. State of the Project

This first annual report on the progress of USAMRDC contract # DADA 17-72-C-2103, titled "Water Content of Stratum Corneum *in vivo*" is written at nine months into the first year of the contract period. In the original proposal ~~(E)~~, research was set down as two 18 month periods. The first 18 month period was to be utilized in the development of appropriate methodology for and the measurement of water content of stratum corneum *in vitro*, and the second 18 month period was to be utilized in applying these *in vitro* methods and findings to the problem of measuring the water content of stratum corneum in intact skin.

At the midpoint of this first eighteen month period, we are pleased with the overall progress of the research project. Some unexpected delays were encountered associated with moving to new laboratory space, delivery of equipment and supplies, and installation of special isolation filters in the laboratory power lines. These problems have now been overcome and although final development, fabrication and validation of *in vitro* testing apparatus was initially delayed by 2-3 months, we have put forth extra effort and are now close to being on ~~our original projected~~ schedule.

Unexpected advances have occurred in some areas of the research project. In collaboration with Dr. Eugen Schibli of the Department of Electrical Engineering, we are developing electrical microcircuits on glass substrates. ~~These devices~~ will be used to measure the electrical impedance of conditioned



samples of stratum corneum simultaneously with the thermal testing. Thus we will be evaluating indirect, nondestructive measures of two unrelated physical properties as practical measures of stratum corneum water content.

The prospect of measuring electrical impedance of the horny layer is very exciting to us for two reasons. First, it offers a potential alternative to the use of thermal methods for non-destructively assessing the water content of intact stratum corneum on the palms and soles, the technical objective of the original contract proposal. Secondly, using microcircuit technology, it is possible to measure electrical impedance of very thin layers, such as normal thickness stratum corneum. If a reproducible, quantitative relationship exists between water content and impedance *in vitro*, then it may be possible to develop a method for determining the water content of intact stratum corneum which would not be limited to areas where the stratum corneum is naturally thick, as thermal methods presently are (1), but which could be used anywhere on the body surface. The first microcircuit will be ready for evaluation sometime in February.

Under the direction of Dr. Colin H. Daly of the Department of Mechanical Engineering, a group of students conducted a pilot study on the breaking strength and elongation of stratum corneum preconditioned to various relative humidities (2). The idea for the student problem was an outgrowth of this project, and we furnished modest amounts of supplies and human tissue for their experiments. While not enough data were obtained in these student

experiments to draw any firm conclusions, the results that were obtained appear to be at variance with published values (3). We hope to be able to encourage more investigation into the problem of the relationship of mechanical properties and water content of stratum corneum.

A manuscript which details the research efforts leading to the development of the current thermal methodology for *in vitro* assessment of stratum corneum water content has been submitted for publication to the Journal of Applied Physiology. (4).

II. Research Progress: Development of Methodology for Determining kpc and k/pc in Isolated Stratum Corneum

In the original research plan, it was pointed out that the first task of the project would be to fabricate an apparatus that would allow accurate and reproducible application of Parker's method (5) and Buettner's method (6,7) for determining k/pc and kpc respectively, to conditioned specimens of stratum corneum. The main effort during this first nine-month period has been in the development, fabrication and validation of such a device. The development of more accurate and reliable instrumentation revealed unanticipated sources of errors in matching the experimental setup to the boundary conditions of Buettner's simple mathematical model which were not seen in initial pilot studies with prototype instrumentation reported in the proposal (1). Fortunately, these problems were noticed and largely solved before we accumulated large amounts of data.

A. Description of Instrumentation

The finalized device is constructed in such a way as to allow determination of both kpc and k/pc without repositioning the specimen. Both sides of the sample can be irradiated; the front side with far-infrared radiation for determination of kpc and the back side with a pulse of light from a flash lamp for determination of k/pc (Figure 1). In the center of the device, the specimen, mounted on an aluminum microscope-size slide, is inserted. The front side of the specimen contacts a small thermocouple bead for determining the temperature rise.

1. Infrared source.

Far infrared radiation is used to heat the sample surface because skin absorptivity of radiant energy at these wave lengths is nearly unity (6). Thus, there is no need for a layer of surface blackening, as would be the case if visible radiation were used. Infrared radiation is generated with a 30 watt reflector bulb whose surface has been heavily coated with flat black paint. The paint layer prevents the transmission of visible radiation, but the heat from the bulb warms the glass surface and paint layer to approximately 520°K. The heated paint layer then serves as the source for far-infrared radiation. To minimize electrical interference pickup by the thermocouple the light bulb is driven by a 130 volt DC power supply. A copper and constantan thermocouple stretched across the surface of the light bulb is used to monitor the paint layer temperature. Infrared

radiation is directed from the bulb through a polished cone which tapers from 9 cm D to 4 cm D over its 10 cm length (Figure 2). The polished cone serves to separate the heat source and the sample and minimize radiation losses from the source. At the 4 cm end of the cone is fastened a camera shutter with a 3.5 cm opening. The camera shutter is operated remotely by a pneumatic device. The other side of the shutter opening is connected to a 4 cm D hollow polished cylinder, 10 cm in length. The surface of the sample with thermocouple in place is fixed in the center of the cylinder end. When the shutter is opened, the sample front surface is irradiated with infrared radiant energy from the bulb surface. The 20 cm total connecting length of the cone and cylinder minimizes the effect of hot air convection currents on the temperature of the sample surface. Exposure of the sample surface is signalled by a photocell mounted inside the 10 cm D cylinder. The photocell is connected to external electronic circuitry for producing a marker pulse on a chart recorder or to trigger an oscilloscope trace sweep (Figure 3).

Heat flux density at the end of the cylinder is measured with a calibrated heat-flow disc (National Instrument Laboratories, Washington, D.C.) which has an output of 36.8 microvolts for each millicalorie/cm²-sec of heat flow in the steady-state. In practice, the heat flow disc is substituted for the sample under test, the shutter is opened, and the heat flow is allowed to attain a steady state (Figure 4).

The front surface of the heat flow disc is previously blackened to insure total absorption of the infrared energy and the back surface is connected to a large metal heat sink. Application of silicone grease between the back of the heat flow disc and the heat sink insures good thermal contact.

The radiant heat flux measured in this way which strikes the sample surface is about 5 millicalories/cm²-sec, a very mild level of heating which results in elevation of sample temperature only 1-5 degrees C, depending on water content, over a several second heating period. This mild heating level was purposely chosen to minimize evaporation of water from the samples during thermal testing.

2. Sample holder and thermocouple temperature measurement.

The test specimens are mounted on aluminum slides which fit precisely into position on a sample holder (Figure 5), so that the specimen is always located in the same way during repeated testing following exposure to various conditioning environments. The thermocouple is also mechanically attached to the sample holder. The sample holder, thermocouple, and slide with specimen in position can be removed as a package from the testing device for inspection of thermocouple placement under an opaque specimen microscope. The whole package can then be reinserted in exactly the same position in front of the infrared cylinder assembly. This insures an invariable flux of infrared radiation on the sample surface.

The sample surface temperature is measured with a copper and constantan thermocouple and a specially designed DC amplifier which minimizes pickup of electrical interference, and has variable gain, offset, and filtering to suit the needs of a particular experiment (Figure 3). This has proven necessary as the time course and maximum temperatures differ in Buettner's method (6,7) and Parker's method (5).

Difficulty has been experienced in obtaining suitable and reproducible thermal contact between the thermocouple bead and the sample surface. The problem is more pronounced on hard surfaces of calibration substances such as glass than on tissue. Several different techniques for securing good thermocouple contact are being evaluated. The first is illustrated in Figure 5a. A fine wire thermocouple (0.001" wire) is centered in the middle of the sample port, and the two leadwires are lightly stretched and the ends connected to binding posts. The rest of the slack in the thermocouple is taken up by two small weights hung between the pairs of supports mounted on either side of the sample port. When the sample is in position, it pushes lightly on the thermocouple wire and thermocouple bead. The final tension is determined by the mass of the weights. As the samples are always positioned in the same way, the thermocouple comes into contact with the sample in exactly the same spot and under the same tension each time it is inserted into the sample holder. The advantages of this technique

are that the positioning of the thermojunction is automatic and extremely reproducible and the contact between the thermojunction and the sample surface is fixed by the mass of the weights. The leadwires close to the thermojunction are also pressed against the sample surface, thus greatly reducing environmental influences on the measured junction temperature. The main disadvantage of this technique lies in the physical nature of the thermocouples. These extremely fine wire thermocouples were chosen because of their fast response characteristics (8); however, they are extremely fragile and susceptible to bending. Small residual twists and "kinks" in the leadwires tend to elevate the thermocouple off of the surface of the sample resulting in poor thermal contact.

A second technique is illustrated in Figure 5b. In this simpler method the thermocouple leadwires are routed in parallel to the sample port. A single right angle bend inward is made in both wires about one cm from the thermojunction. The thermojunction then presses directly inward on the sample with affixed spring force, determined by the characteristics of the leadwire. This second technique has been used with both the fragile 0.001" (50 gauge) thermocouple and a more substantial one made from 30 gauge thermocouple wire. The latter, although exerting firmer contact with the sample surface has a greater response time; 15 msec for 63% response as compared with 2-3 msec for the .001" thermocouples. Because variations in thermal contact resis-

tance could result in serious errors in estimating kpc and k/pc we favor the use of the larger thermocouple. The face of the thermojunction is ground flat to insure a large area of sample contact. As the samples of stratum corneum are usually 1-2 mm thick, we anticipate no restrictions on the measurements because of the slower response time. We have developed an electronic compensator for decreasing the response time if thin samples of horny layer are encountered and a faster response time is needed (Figure 6). In using the compensator, the response time of the thermocouple is first determined by rapid immersion in warm water (Figure 6a). The response characteristics of the thermocouple can be well approximated by an electronic analog of a first order system where the 63% response time is equal to the product of R and C (Figure 6b). When the output of this first order system is fed to inverse electronic circuitry, the original input is restored (Figure 6c). When the output from the thermocouple is applied to the compensator and RC adjusted to match the measured response time of the thermocouple, the response to immersion is modified, as shown in Figure 6d, so that the thermocouple appears to respond about 3 times faster. This decrease in response time, however, is accompanied by an increase in "noise" in the tracing, so that the compensator is not used unless the faster response time is necessary.

3. Flash source.

In Parker's method (5), thermal diffusivity (k/pc) is determined from the transit time from the back to front surface of a thermal wave generated from a flash. In the present experiments a hole is drilled in the back of the microscope slide and the rear surface of the sample is blackened. The flash is directed on this blackened rear surface and the subsequent temperature changes of the front surface are recorded with the previously described thermocouple arrangement. This test immediately follows the kpc experiment; the sample is not moved out of position between the two tests. The flash pulse is generated by a conventional AG-1 type flash bulb. We have found these flash bulbs to be a satisfactory and more economical alternative to the use of a xenon flash, as was originally proposed.

B. System Performance, Preliminary Evaluation

1. Surface heating with infrared radiation.

Figure 7 shows the capability of both slow and fast surface temperature measurement during infrared heating. The test samples were two pieces of pyrex glass 0.104 cm thick and 1.30 cm thick respectively. The glass surfaces were blackened with flat black paint to insure total absorption of the infrared radiation. Radiation intensity was the same in both experiments. The time course of temperature change was quite different for the two samples over a 16 sec heating period (Figure 7a). Buechner's method (6,7) predicts that

the surface temperature will rise according to the following relationship:

$$T_x(t) = \frac{2 H_0 \sqrt{t}}{\sqrt{\pi} kpc}$$

where $T_s(t)$ is the surface temperature rise with time, in $^{\circ}\text{C}$, H_0 is the heat flux density in $\text{cal-cm}^{-2}\text{-sec}^{-1}$, and kpc is the product of thermal conductivity, density, and heat capacity with units $\text{cal}^2\text{-cm}^{-4}\text{-deg C}^{-2}\text{-sec}^{-1}$. However, successful application of Buettner's method depends upon the experimental technique meeting certain mathematical boundary conditions. One boundary condition is that the sample be "infinitely" thick. In practical terms, this means that the sample must be thick enough so that heat conducted from the surface of the sample does not reach the back surface until after the experimental observations are made. For times greater than one second, the thin glass sample clearly violates this condition, resulting in a different time course of temperature change than the thick sample. With our instrumentation it is possible to obtain useful information from the thin sample by examining the smaller temperature changes occurring at the onset of heating. Figure 7b shows data from identical experiments repeated on the same samples, where the temperature rise was measured on an expanded time scale. Identical time-temperature relationships were observed during the first 0.4 sec of heating. A tabulated value for

kpc of glass is 1.17×10^{-3} (9), while the reported value for full thickness human skin is 1.1×10^{-3} (10). This study will involve the use of stratum corneum as thin as 0.1 cm. Thus, the use of 0.1 cm thick glass is a reasonable test material for validating Buettner's method under our experimental conditions.

2. Surface heating from flash irradiations of the back surface.

Figure 8 shows a test result of front surface temperature elevation from a flash bulb fired 10 cm from the rear surface. The sample was the same thin piece of glass (0.104 cm) used in the previous experiment. The back surface of the glass sample was blackened to insure total absorption of the flash irradiation. The thermal wave diffused through the sample reaching a peak on the front surface in about 0.8 sec. According to Parker's theory (5) modified by Taylor (11) to correct for finite pulse time and heat loss affects the thermal diffusivity (k/pc) is given by the equation:

$$1.37 \frac{L^2}{(\pi)^2(t_{\frac{1}{2}})}$$

where k/pc is the thermal diffusivity in $\text{cm}^2\text{-sec}^{-1}$, L is the sample thickness in cm, and $t_{\frac{1}{2}}$ is the time required for the temperature to reach one-half the peak magnitude in seconds. In this test $t_{\frac{1}{2}}$ was 0.30 sec. When substituted into the above equation, k/pc was calculated to be 5.0×10^{-3} . An average reported value for glass is 5.8×10^{-3} ,

but this value is variable depending on the exact composition of the glass (9).

C. Remaining Problems of Instrument Development and Evaluation

Some unresolved problems remain before the two thermal methods can be applied confidently to samples of stratum corneum. Our measured values for kpc in the thick samples of glass are about one-fourth the expected value. We have confidence in the method as others have reported its successful application (e.g. 7,10,12). We have verified that the problem is not related to thermocouple size or response time. Our first suspicion is the heat flux measurement. Currently we are using a heat flow disc, a device originally designed to measure heat exchange between the body surface and environment. We have modified this device by painting its surface black to measure radiant heat flux in this study. Since calculated kpc varies as the square of heat flux density, small errors in measured radiant flux will result in larger errors in estimating kpc. A second calibrated method for determining radiant heat flux must be obtained.

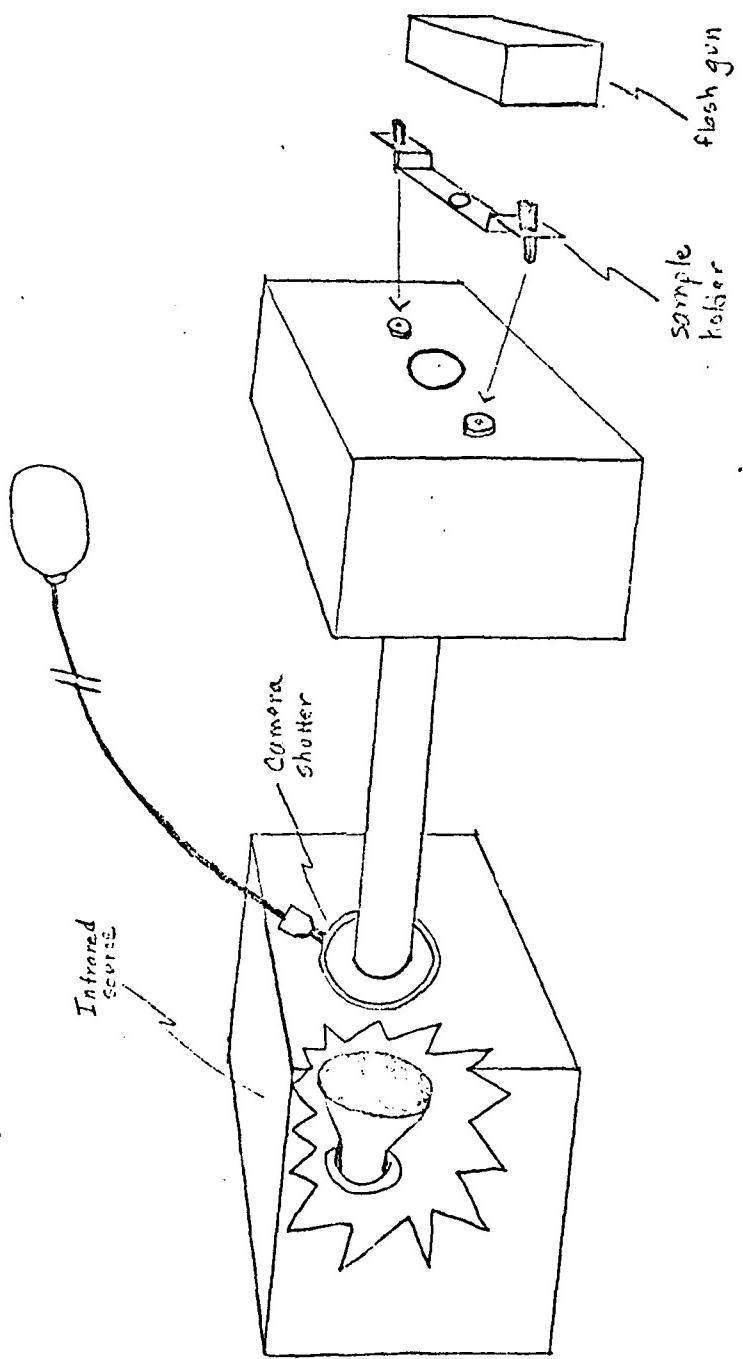
Currently we are using uncalibrated laboratory materials (glass, rubber, etc) and average tabulated values for k, p, and c of these materials (9). Some materials are listed with a fairly wide range of variation in the value of one or more of these constants. Errors are compounded, of course, when the product of the three, kpc is calculated. Additional tests of both methods in our hands must be made with glass samples and other materials whose thermal conductivity, density, and heat capacity have been carefully verified using independent techniques.

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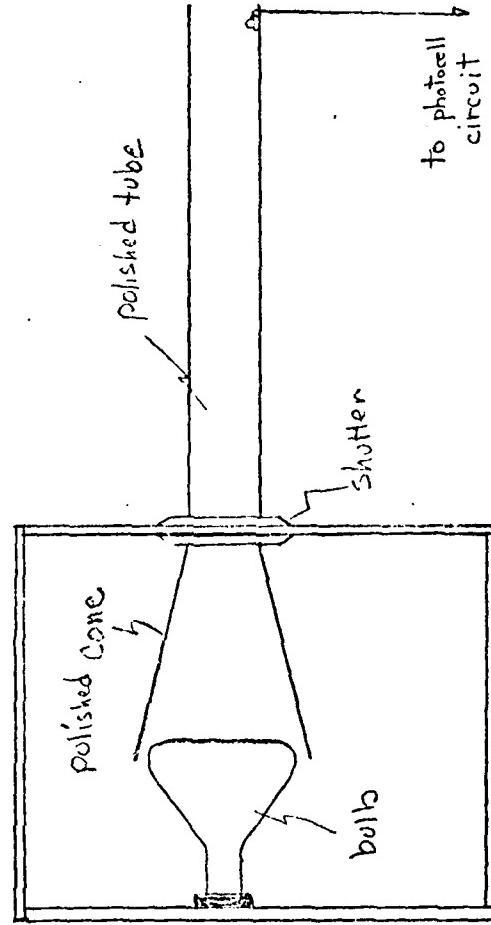
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Overall View of Instrument

FIGURE 1

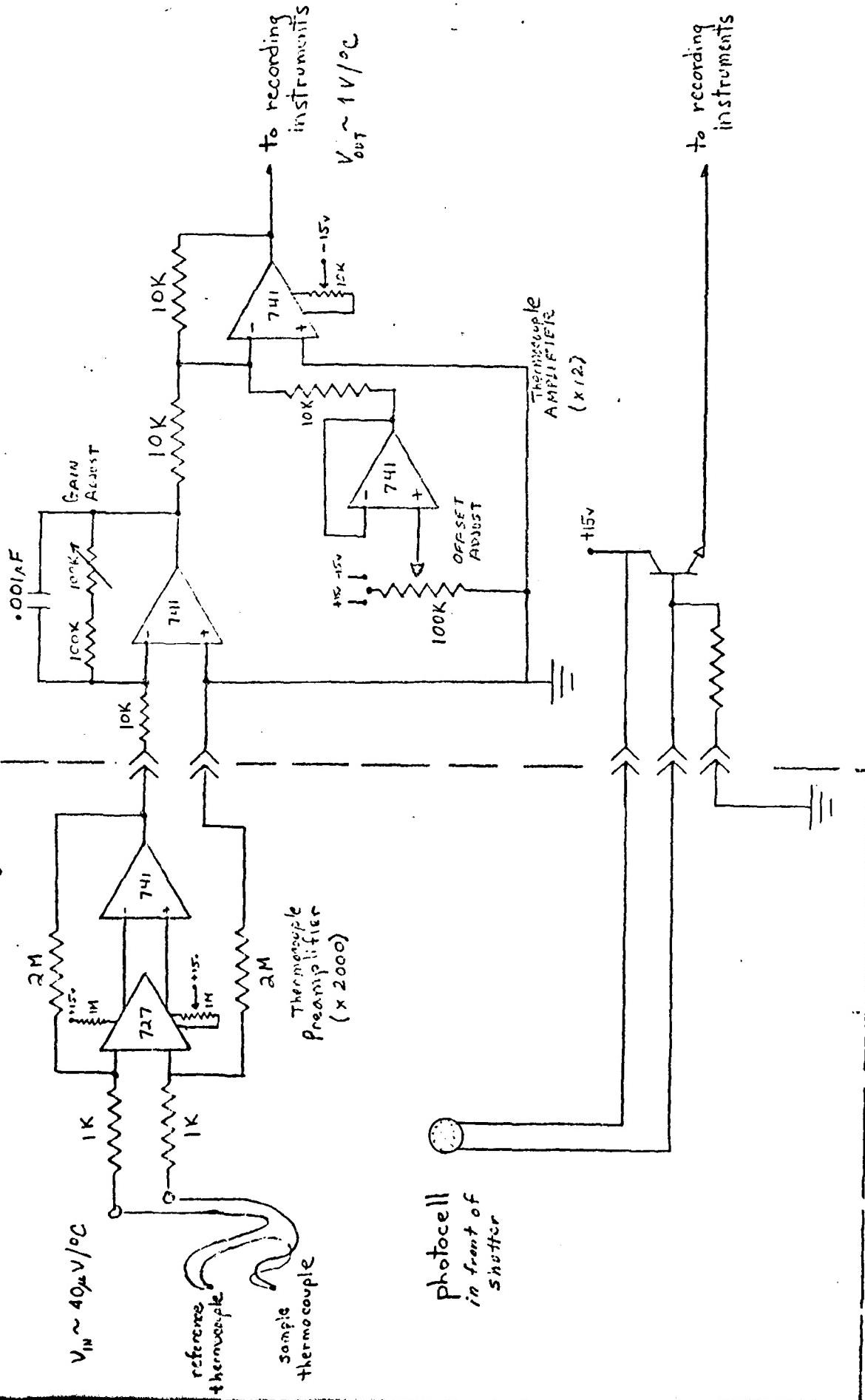


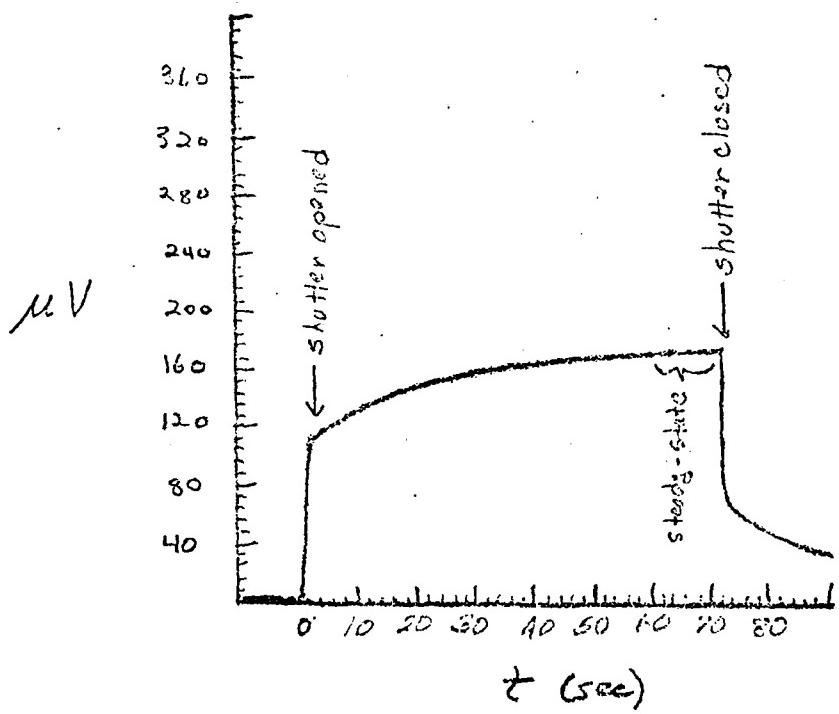
Infrared Source

Figure 2

rf - shielded room

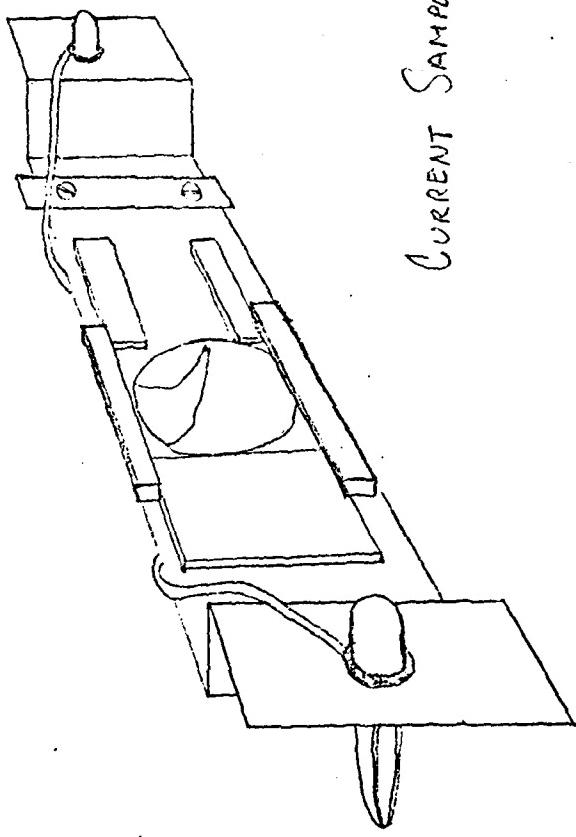
Main instrumentation rack





OUTPUT FROM HEAT FLOW DISC
 $(1\mu V = 2.72 \times 10^{-5} \text{ cal-}cm^{-2}\text{-sec}^{-1})$

FIGURE 4



CURRENT SAMPLE HOLDER

Two methods of contacting sample and thermocouple

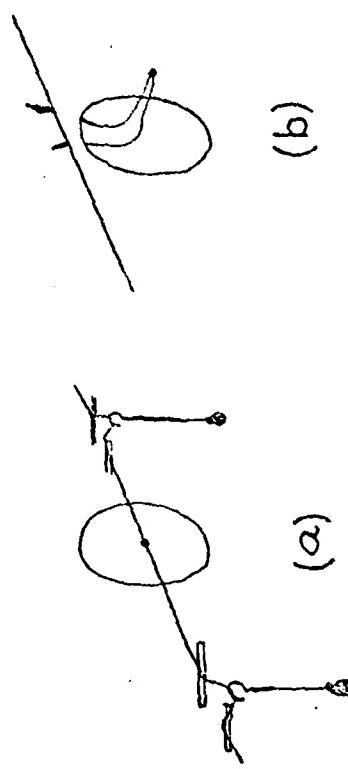
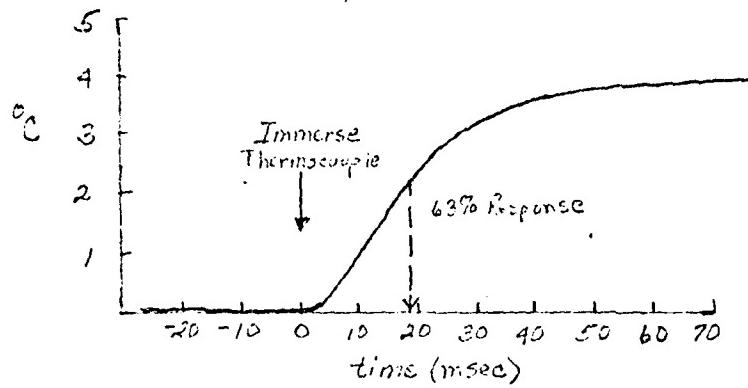
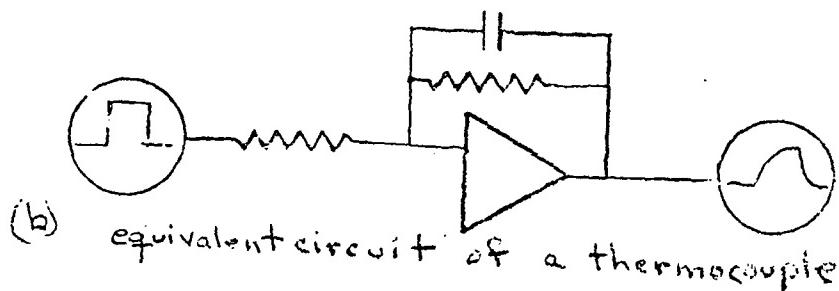


FIGURE 5

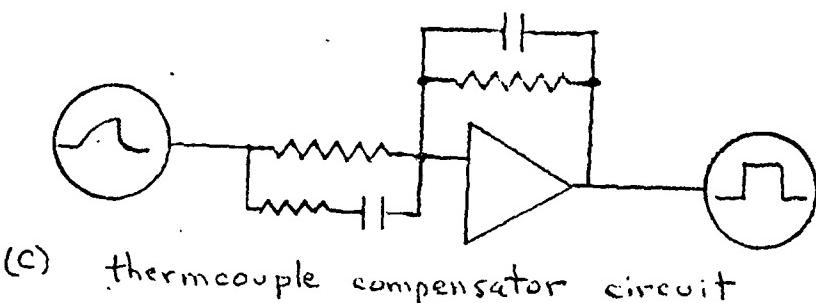
FIGURE 6



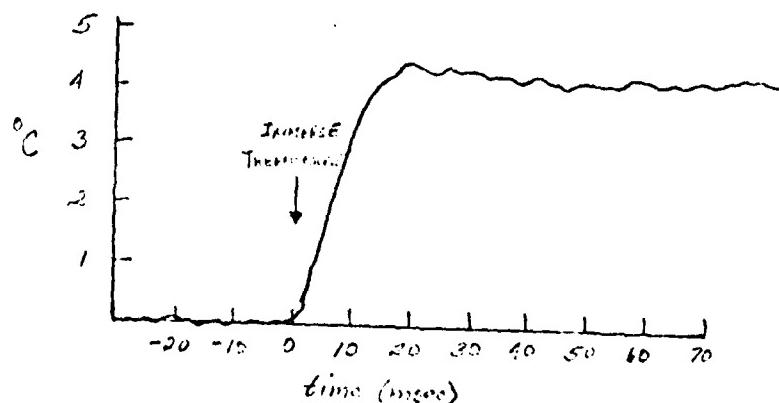
(a) 30 gauge thermocouple response to water immersion



(b) equivalent circuit of a thermocouple

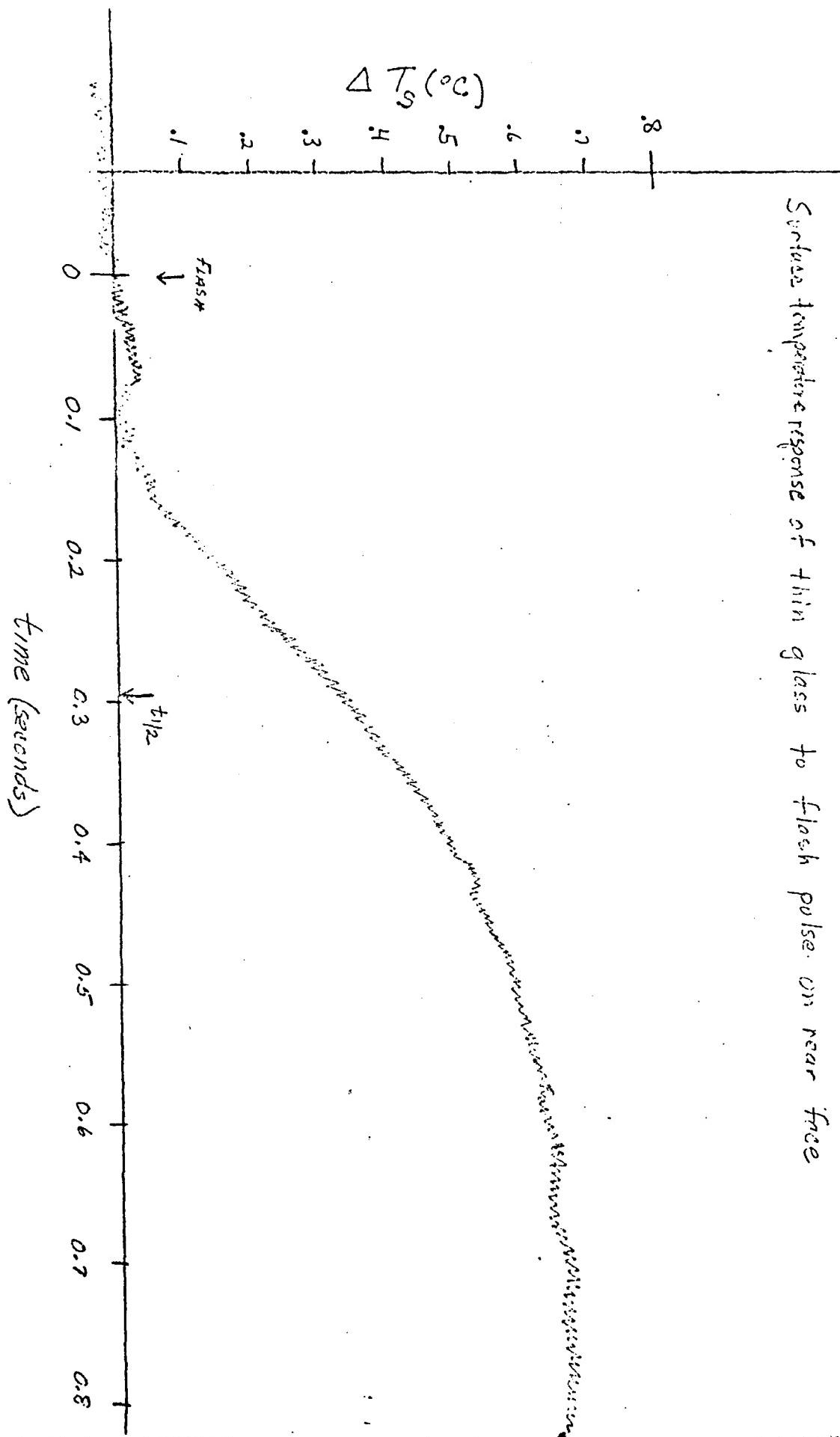


(c) thermocouple compensator circuit

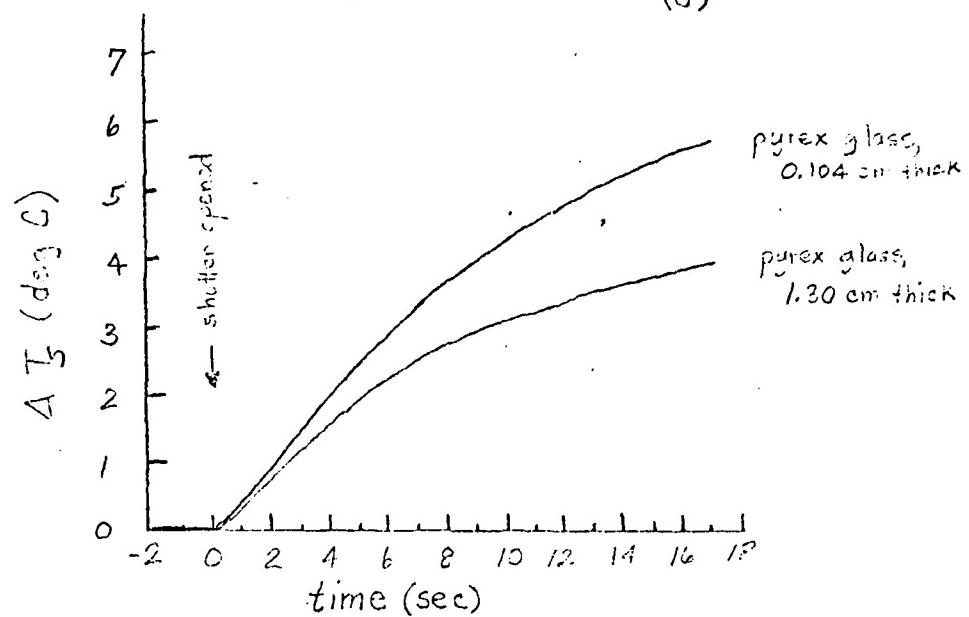


(d) compensated thermocouple response to water immersion

Surface temperature response of thin glass to flash pulse on rear face



(a)



Surface temperature response of glass to
infrared surface heating

(b)

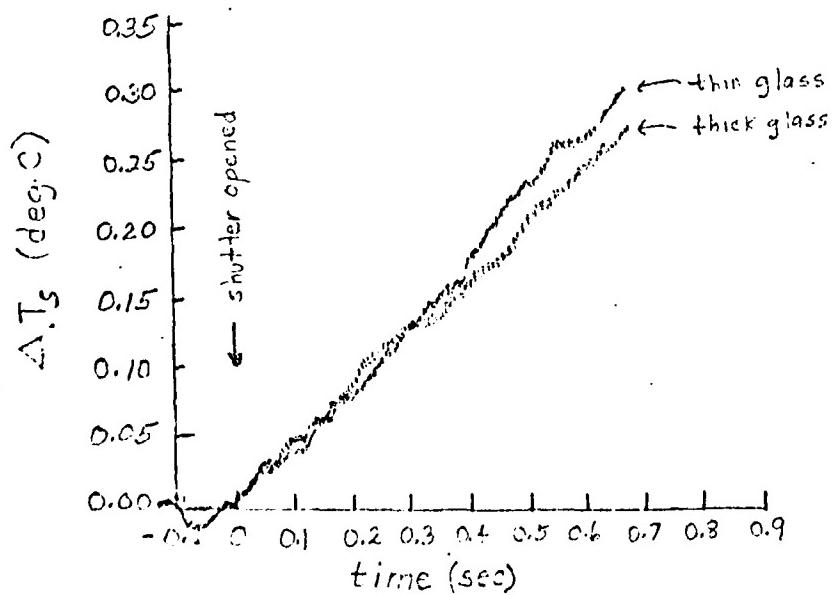


FIGURE 7